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New insight into the substrate binding of dye-linked L-proline dehydrogenase

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Introduction

Dye-linked L-proline dehydrogenase (LPDH) catalyzes the oxidation of L-proline to Δ^1-pyrroline-5-carboxylate with reduction of 2,6-dichloroindophenol. The enzyme is supposed to function as the mediator of electron transfer from an L-proline into the electron transfer system. Two different types of LPDH, PDH1 and PDH2, have been identified in the anaerobic hyperthermophile *Pyrococcus horikoshii*. PDH1 is a heterooctameric complex (α4β4) that also contains FAD, FMN, Fe and ATP, while PDH2 is a heterotetrameric complex (αβγδ) composed of an L-proline dehydrogenase, an NADH dehydrogenase, a ferredoxin-like protein and a protein of unknown function. We have previously solved the three-dimensional structure of the PDH1 [1], which we found to be a unique diflavin dehydrogenase containing a novel electron transfer system. Here we report the crystal structure of a third type of LPDH (ApeLPDH), found in the aerobic hyperthermophilic archaeon *Aeropyrum pernix*, whose structure (homodimer) is much simpler than those of previously studied LPDH. This is the first description of an LPDH structure with L-proline bound, and it provides new insight into the substrate binding of LPDH.

Materials and Methods

Selenium multiple-wavelength anomalous dispersion data for selenomethionyl ApeLPDH and single-wavelength (1.0 Å) data for a deletion mutant lacking C-terminal Leu428 (ΔL428) were collected on the beamline 5A and NW12A at the Photon Factory. The data were processed using HKL2000 and the CCP4 program suite.

Results and Discussion

The overall fold of the ApeLPDH subunit showed similarity to that of the PDH1 β-subunit, which is responsible for catalyzing L-proline dehydrogenation. However, the situation at the subunit-subunit interface of the ApeLPDH was totally different from that in PDH1 [2]. The presence of additional surface elements in the ApeLPDH contributes to a unique dimer association. In addition, the C-terminal Leu428, which is provided by a tail extending from the FAD-binding domain, shielded the active site, and an L-proline molecule was entrapped within the active site cavity (Figure 1). Because L-proline was not present during crystal growth, this molecule might have originated in the *E. coli* cells and was then retained throughout the protein purification.

The Km of a ΔL428 for L-proline was about 800 times larger than the Km of the wild-type enzyme, though the kcat values did not differ much between the two enzymes. In the ΔL428 structure, we found no L-proline molecule in the active site. Moreover, we superimposed FAD-binding domain of the ApeLPDH onto the equivalent residues of ΔL428, and found that substrate-binding domain of ApeLPDH was rotated about 10° relative to the corresponding domain of ΔL428. This indicates that L-proline incorporation leads to a rotation of the substrate-binding domain. Therefore, the ApeLPDH structure assumes a closed conformation and the ΔL428 structure assumes an open one. The C-terminal chain of the ΔL428 was about 13° rotated clockwise compared with the corresponding residues of ApeLPDH, and this results in the active site cavity being solvent-accessible (Figure 2). These results strongly suggest that the function of the C-terminal Leu428 is to provide a solvent-inaccessible environment for the enzyme reaction, as well as to hold the substrate properly within the active site.

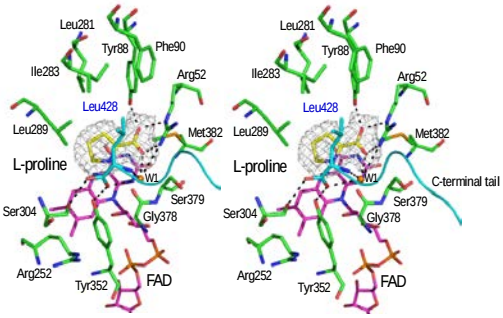

Figure1 Stereographic close-up of the L-proline-binding site in ApeLPDH

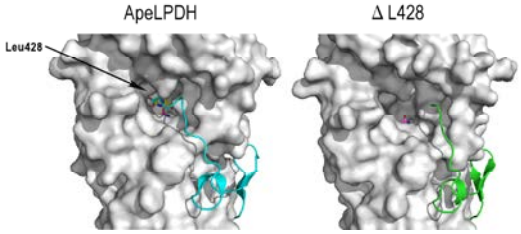

Figure 2 Close-up views of the active site and C-terminal region

References

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